Sains Malaysiana 45(2)(2016): 195–200

Antioxidative Activities and Polyphenolic Content of Different Varieties of Malaysian Young Corn Ear and Cornsilk

(Aktiviti Antioksida dan Kandungan Polifenolik bagi Varieti Berbeza Jagung Muda dan Sutera Jagung Malaysia)

HO, Y.M. WAN AMIR NIZAM, W.A. & WAN ROSLI, W.I.*

ABSTRACT

This study aimed to determine the antioxidant capacity, total polyphenolic and total flavonoid content of Malaysian young corn ear (YCE) and its cornsilk (CS) of different varieties. Three different varieties of YCE and its CS locally grown in Malaysia were chosen namely Big Fruit, Thai Supersweet and Bi-color. CS Bi-color displayed the highest total phenolic content (143.58 mg GAE/g extract) while CS Thai Supersweet exhibited the highest total flavonoid content (26.63 mg CAE/g extract). Meanwhile, YCE of Bi-color variety demonstrated the highest total polyphenolic and flavonoid content (92.64 and 18.14 mg CAE/g extract, respectively) compared to other YCE varieties. At 800 µg/mL, CS of Bi-color (93.82%) recorded the strongest electron donor due to higher DPPH scavenging activity, followed by CS Thai Supersweet (92.87%), YCE Bi-color (41.94%), YCE Thai Supersweet (28.87%), CS Big Fruit (28.87%) and YCE Big Fruit (21.38%). For FRAP, CS of Bi-color showed the highest reducing power activity (65.46%) among all the crude extracts. There is a significant correlation between total polyphenolic content, DPPH free radical scavenging activity and FRAP of YCE and CS of different varieties. In summary, CS extracts are the potential ingredient to be applied in food industries and at the same time reducing agriculture wastage.

Keywords: Antioxidant capacity; cornsilk; different varieties; young corn ear

ABSTRAK

Kajian ini bertujuan menentukan kapasiti antioksidan, kandungan jumlah polifenolik dan flavonoid dalam varieti berbeza jagung muda (YCE) dan sutera jagung (CS) Malaysia. Tiga varieti berbeza YCE dan CS yang ditanam di Malaysia telah dipilih iaitu Big Fruit, Thai Supersweet dan Bi-color. CS Bi-color menunjukkan kandungan jumlah polifenol paling tinggi (143.58 mg GAE/g ekstrak) manakala CS Thai Supersweet menunjukkan kandungan jumlah flavonoid yang tertinggi (26.63 mg CAE/g ekstrak). Sementara itu, YCE varieti Bi-color menunjukkan kandungan jumlah polifenol dan flavonoid tertinggi (masing-masing 92.64 dan 18.14 mg CAE/g ekstrak) berbanding varieti YCE yang lain. Pada 800 µg/mL, CS Bi-color (93.82%) merekodkan pendermaan elektron terkuat disebabkan aktiviti perencatan DPPH yang tinggi, diikuti oleh CS Thai Supersweet (92.87%), YCE Bi-color (41.94%), YCE Thai Supersweet (28.87%), CS Big Fruit (28.87%) dan YCE Big Fruit (21.38%). Untuk FRAP, CS Bi-color menunjukkan aktiviti kuasa penurunan paling tinggi (65.46%) antara semua ekstrak mentah. Terdapat korelasi signifikan antara kandungan jumlah polifenolik, perencatan radikal bebas DPPH dan FRAP bagi varieti berbeza YCE dan CS. Secara kesimpulannya, ekstrak CS berpotensi sebagai ramuan untuk diaplikasikan dalam industri makanan dan dalam masa yang sama dapat mengurangkan sisa pertanian.

Kata kunci: Jagung muda; kapasiti antioksidan; sutera jagung; varieti berbeza

INTRODUCTION

Herbs or botanicals are one of the primeval fundamental elements responsible in sustainable health, which constitutes the basic platform of modern medicines (Wan Rosli et al. 2010). Exploration of value-added stable diets remains in pursuit of researchers all around the world as concern in the importance of dietary phytochemicals in the prevention of several diseases increased.

Young corn ear (YCE) is harvested as young, unfertilized ear either when the silks have not emerged or the silks have just emerged. Cornsilk (CS) is a collection of the stigmas which is the fine, soft and yellowish threads from the female flowers of the maize plant. It is found inside the husks of corn with approximately 4 to 8 inches long and taste mild sweet. CS also known as *Maydis stigma* or *Zea mays* hairs is used for traditional herbal remedy before the plant is pollinated (Maksimovic et al. 2005). CS is highly treasured as traditional medicine to treat disease or symptoms related to urinary system (Maksimovic & Kovacevic 2003). The main essential phytochemicals occurred in CS are silicon, B vitamins, para aminobenzoic acid (PABA) and trace amounts of iron, zinc, potassium, calcium, magnesium and phosphorus (Fleming 2000).

CS has been reported to have polyphenol compounds which can be considered as potential herbal drug (Maksimovic & Kovacevic 2003). Thus, YCE and its CS which were found in abundance and also easily

accessible agricultural by-products can be potentially used as an alternative food additive or as natural dietary supplement to boost up health, as well as to conserve the sustainability of environment by optimizing the use of agricultural waste. Other than that, this may also increase side income for the farmers indirectly and initiate new field to the development of Small and Medium Enterprises (SMEs). However, there were so many studies which investigated the phytochemical and nutrient composition of CS around the world, but little study has been carried out in Malaysia using Malaysian corn varieties. Thus, holistic and multidisciplinary research on the YCE and its CS is needed to provide the public with scientifically sound and accurate information. The objective of this study was to determine the antioxidant capacity, total phenolic and total flavonoid content in YCE and CS of different varieties which is locally available in Malaysia.

Materials and Methods

PLANT MATERIALS

There were six samples of young corn ears (YCE) and its cornsilk (CS) of three different varieties of corn grown in Kelantan state of Peninsular Malaysia used in this study. They were Big Fruit (BF), Supersweet (SS) and Bi-Color (BiCo) varieties. These varieties were purchased from local farmers in Bachok District, Kelantan state of Peninsular Malaysia. Upon arrival at Nutrition Laboratory of The School of Health Sciences, Universiti Sains Malaysia, both YCE and CS were separated from the cob. Both YCE and CS were then dried at 55°C in the oven (Memmert, Germany) to achieve 5-10% (w/w) of moisture content. Laboratory blender (Waring, USA) was used to grind dried YCE and CS into powder form separately. Powder of YCE and CS was sieved with 80 mesh size sieve shaker (Retsch, Germany) and stored in the air tight Duran bottle before refrigerated.

CHEMICALS AND REAGENTS

Gallic acid, (+)-catechin, butylated hydroxytoluene (BHT), linoleic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Sigma (St. Louis, MO, USA). Folin-Ciocalteau reagent, potassium ferricyanide ($K_3Fe(CN)_6$, iron (III) chloride anhydrous ($FeCl_3$) and other chemicals were purchased from Merck (Darmstadt, German). Other chemicals and solvents were of analytical grade standards.

EXTRACTION PROCEDURE

Exactly 15 g of YCE powder and CS powder from different varieties were extracted with 300 mL absolute ethanol with a ratio of 1:4 using Soxhlet extraction method. Both YCE and CS powder were filled in the thimble and placed in the extractor section. Heating mantel (Thermo Scientific, EM 1000/CE) was set to scale 4 to heat the ethanol in the round bottom flask which attached to the main Sohxlet extractor. Once the ethanol evaporated through by-pass tube and filled over the thimble, the extraction was start on. Then the ethanol which carrying polyphenolic compounds gone through the siphon tube and plunged back into the round bottom flask. As the extraction process continued, the colour of mixture in the extractor became darker. The extract was then vacuumed evaporated (50°C) by using rotary evaporator (Eyela OSB-2100, USA) and oven dried overnight.

DETERMINATION OF PERCENTAGE OF EXTRACT RECOVERY

The yield of the extract obtained was reported as percentage and was calculated as:

$$\text{Percentage of recovery (\%)} = \frac{W_a - W_b}{\text{Weight of dry plant used (g)}} \times 100,$$

where W_a is the weight of aluminium dish (g) +crude extract (g); and W_b is the weight of aluminium dish (g).

DETERMINATION OF TOTAL PHENOLIC CONTENT

The phenolics content of each extract was determined by using Folin-Ciocalteau method as described by Kaur et al. (2008). One mL of the extract (1000 µg/mL) and 0.5 mL of Folin-Ciocalteau reagent (1:1) were added into a 10 mL volumetric flask. The solution was swirled and added with 1.5 mL of sodium carbonate (20% w/v) and raised with distilled water. The solution was left to stand at room temperature for 2 h in the dark area. The absorbance was recorded at 765 nm using UV-Vis spectrophotometer (Varian, USA) against blank. The phenolics content was compared with gallic acid standard curve and expressed as (mg GAE/g crude extract).

The TPC was calculated by comparing the absorbance with the gallic acid calibration curve according to the formula:

$$\text{TPC (mg/g)} = C \times V / g,$$

where C is the concentration of the gallic acid equivalent from standard curve (µg/mL); V is the volume of the extract used (mL); g is the weight of extract (g); and the contents were expressed as gallic acid equivalent (mg GAE/g).

DETERMINATION OF TOTAL FLAVONOID CONTENT

In this test, 0.25 mL of the extract (1000 µg/mL) was added into a bottle followed by the addition of 75 µL of sodium nitrite (5% w/v). The mixture was reacted for 6 min after which 150 µL of aluminum chloride (10% w/v) was added. The mixture was left to react for another 5 min before added with 0.5 mL of NaOH (1 M). The solution was raised to 2 mL with distilled water. The absorbance of the sample was measured at 510 nm by using UV-Vis spectrophotometer (Varian, USA). Catechin was used as a standard and the flavanoid content was expressed as mg CAE/g crude extract (Ozsoy et al. (2008).

The TPC was calculated by comparing the absorbance with the catechin calibration curve according to the formula:

$$\text{TFC (mg/g)} = C \times V / g,$$

where C is the concentration of the catechin equivalent from standard curve (μg/mL); V is the volume of the extract used (mL); g is the weight of extract (g); and the contents were expressed as catechin equivalent (mg CAE/g).

DETERMINATION OF DPPH FREE RADICAL SCAVENGING ACTIVITY

The free radical scavenging activity of the extract was performed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable free radicals as established by Vimala et al. (2003). Plant extract (4 mL) (50 to 800 μg/mL) was mixed with 1 mL of DPPH solution (1 mM). For the negative control, 4 mL of methanol and 1 mL of DPPH solution were used while BHT was used as the positive control. The reaction mixture was left in the dark for 30 min at room temperature before the absorbance was recorded at 520 nm by using UV-Vis spectrophotometer (Varian, USA). The free radical scavenging activity of the sample of each concentration was expressed by percentage of inhibition and was calculated as follow:

$$\text{Scavening activity}\,(\%) = \frac{(\text{Abs}_{\text{negative control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{negative control}}} \times 100.$$

DETERMINATION OF FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

One mL of the extract (1000 μg/mL) or BHT (50 to 400 μg/mL) was filled into separate test tubes. Phosphate buffer (0.2 M, pH6.6) (2.5 mL) and potassium ferricyanide (1% (w/v) K_3FeCN_6) (2.5 mL) were then added into the tube. The mixture was incubated at 50ºC in a water bath for 20 min. After that 2.5 mL of trichloroacetic acid (TCA) (10% w/v) was added and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer (2.5 mL) of the solution was pipetted out and mixed with 2.5 mL of distilled water in another tube. Subsequently, 0.5 mL of iron (III) chloride (0.1% w/v) was added into the TCA solution and left to react for 10 min. The colour changes of the mixture were measured spectrophotometrically at 700 nm (Varian, USA). The antioxidant activity was expressed as percent and was calculated using formula $(1-(1-A_{sample}/A_{control})) \times 100$ (Kaur et al. 2008).

STATISTICAL ANALYSIS

All experiments were conducted in triplicate and data expressed as mean ± standard deviation. The analysis of variance (ANOVA) and Tukey's multiple comparison were considered significant at $p<0.05$ (SPSS Version 20, SPSS Inc. Chicago). Correlation coefficients (R) and coefficients of determination (R^2) were also calculated (SPSS Version 20, SPSS Inc. Chicago).

RESULTS AND DISCUSSION

PERCENTAGE OF EXTRACT RECOVERY

Apparently, CS samples exhibited significantly higher percentage of extract recovery ($p<0.05$) compared to YCE samples. The highest recovery of CS was BiCo (47.79%) followed by SS (45.25%) and BF (43.08%) varieties (Table 1). Among all varieties of CS, there were no significant different in percentage of extract recovery. All CS samples recorded significantly higher percentage of extract recovery if compared to YCE samples. Besides that, yield of YCE of different varieties also followed the same trend as shown in CS.

TABLE 1. Percentage of extract recovery of different varieties of YCE and CS

Extract	Yield (%)
YCE-BF	15.76±0.58^{c}
YCE-SS	16.53±1.18^{bc}
YCE-BiCo	28.13±3.53^{b}
CS-BF	43.08±7.52^{a}
CS-SS	45.25±4.47^{a}
CS-BiCo	47.79±4.39^{a}

$^{a-c}$ The superscript with different letter within column is different statistically at $p<0.05$

The percentage of extract recovery from the present study was higher than the previous studies done on CS with the same solvent but differ in term of dried sample used (Nurhanan & Wan Rosli 2013). If too little dried sample was used, crude extract yield may also decreased because relatively higher amount of the extract will be wasted as it was not able to remove from the glassware. Besides that, the possible reason for the higher percentage of recovery in this particular plant may due to the genetic traits that inherited from the parent plants and differences in growing environment. The percentage of CS extract recovery from the present study was also higher than the previous article where the ethanolic extract yield of durum wheat bran was 12.1%, buckwheat hulls was 23.8% and oat hulls was 0.035% (Moure et al. 2001).

TOTAL PHENOLIC CONTENT

The total phenolic content (TPC) of the crude extracts of YCE and CS were varied from 79.61-92.64 to 86.26-143.58 mg GAE/g extract, respectively (Table 2). Among all samples, BiCo extracts of CS shown remarkably higher TPC (143.58 GAE/g) than others varieties (86.26-136.32 GAE/g). All CS samples recorded significantly the highest content of TPC than YCE. Kalt (2005) found that there was substantial genetic variation among fruit and vegetable cultivars.

Different variety of sweet corn may possess different value of antioxidant activity due to genetic variation. White corn was found to contained higher concentrations of total polyphenolics than both American and Mexican blue corn genotypes (Del Pozo-Insfran et al. 2006).

TABLE 2. TPC of different varieties of YCE and CS extracts

Extract	TPC (mg GAE/g extract)
YCE-BF	79.61±3.81^{c}
YCE-SS	82.85±2.66^{bc}
YCE-BiCo	92.64±4.51^{b}
CS-BF	86.26±4.70^{bc}
CS-SS	136.32±3.95^{a}
CS-BiCo	143.58±0.90^{a}

$^{a-c}$The superscript with different letter within column is different statistically at $p<0.05$

TOTAL FLAVONOID CONTENT

The flavonoid content (TFC) of the crude extracts ranging from 9.31 to 26.63 mg CAE /g extract. CS-SS displayed the highest TFC (26.63 mg CAE /g extract) and was significantly higher than others CS-BF (14.66 mg CAE/g extract) and CS-BiCo (18.14 mg CAE/g extract) (Table 3).

The colour of extract may also inferred TFC of the YCE and CS as the bright green colour of CS showed to contain higher TFC in the present study. CS which formed surrounded the YCE may have received more sunlight exposure than YCE and thus possess higher TFC value. Zhishen et al. (1999) found that fresh leaves allow the largest amount of flavonoids to be extracted if compared to air-dried and oven dried leaves possible due to prolong storage and high temperature treatment. Thus, extraction method which involve fresh sample should be consider in future study.

DPPH FREE RADICAL SCAVENGING ACTIVITY

DPPH scavenging activity (%) has increased with the increased concentration of the extract in all samples. At the highest concentration (800 µg/mL) tested, CS-BiCo (93.82%) exerted the stronger electron or hydrogen donor due to the higher DPPH scavenging activity, followed by CS-SS (92.87%), YCE-BiCo (41.94%), YCE-SS (28.87%), CS-BF(28.87%) and YCE-BF (21.38%) (Table 4).

TABLE 3. TFC of different varieties of YCE and CS extracts

Extract	TFC (mg CAE/g extract)
YCE-BF	9.31±2.91^{b}
YCE-SS	10.65±1.72^{b}
YCE-BiCo	14.41±4.36^{b}
CS-BF	14.66±6.71^{b}
CS-SS	26.63±3.09^{a}
CS-BiCo	18.14±2.37^{a}

$^{a-b}$The superscript with different letter within column is different statistically at $p<0.05$

Pigmented genotypes showed strong antioxidant capacity with the DPPH among the 18 genotypes of Mexican corn (Lopez-Martinez et al. 2009). The lowest values were obtained from the creamy yellow genotype while all of the samples exhibited an increase in DPPH from the early stage to the mature stage (Harakotr et al. 2014). BiCo variety was the hybrid of white and yellow corn which may indirectly inherited the trait of increased antioxidant capacity from either or both parent breed as evident by the highest DPPH free radical scavenging activity than BF and SS varieties. This DPPH activity of the present study is comparatively higher than brown lentil husk which had DPPH radical scavenging activity of 0.63 µg/g (Moure et al. (2001).

FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

CS extract of BiCo showed the highest reducing power (65.46%) among all crude extracts. When comparison was carried out among different varieties, reducing power displayed by all YCE extracts were lower than their corresponding CS of same variety. In addition, FRAP values of YCE-SS (35.81%) and YCE-BiCo (41.39%) were significantly lower than CS extract of same variety which were 59.92 and 65.46%, respectively (Table 5).

Xu and Chang (2007) confirmed that the FRAP value was affected by the extracting solvents and ethanol was suggested as a better solvent than acetone for yellow pea, green pea and chickpea extract. There was also an inverse relationship between the particle size of sample and antioxidant activities based on FRAP assay as decreased particle size will increase the antioxidant activity in FRAP (SunMi et al. 2012). Higher FRAP value exhibited by BiCo variety in this study may be benefit from the white trait it had inherited.

TABLE 4. DPPH free radical scavenging activity of different varieties of YCE and CS extracts

Extract	Concentration (µg/mL)				
	50	200	400	600	800
YCE-BF	4.41±1.68^{b}	9.52±1.57^{c}	13.91±1.54^{e}	17.42±1.46^{d}	21.38±1.40^{d}
YCE-SS	4.64±1.68^{b}	4.73±1.63^{d}	18.44±1.40^{d}	25.48±1.28^{c}	29.87±1.24^{c}
YCE- BiCo	6.38±1.65^{b}	12.08±1.57^{c}	23.58±1.35^{c}	39.37±1.10^{b}	41.94±1.11^{b}
CS-BF	4.90±1.68^{b}	11.14±1.58^{c}	15.66±1.49^{de}	23.16±1.38^{c}	28.87±1.33^{c}
CS-SS	18.44±1.44^{a}	56.05±0.82^{b}	87.37±0.42^{b}	92.71±0.15^{a}	92.87±0.13^{a}
CS-BiCo	5.96±1.63^{b}	75.67±0.39^{a}	93.32±0.07^{a}	93.65±0.07^{a}	93.82±0.20^{a}

$^{a-c}$The superscript with different letter within column is different statistically at $p<0.05$

TABLE 5. FRAP of different varieties of YCE and CS extracts

Extract	FRAP (%)
YCE-BF	35.81±0.37[d]
YCE-SS	35.81±0.16[d]
YCE-BiCo	41.39±2.90[c]
CS-BF	38.90±0.98[cd]
CS-SS	59.92±0.73[b]
CS-BiCo	65.46±1.71[a]

[a-d] The superscript with different letter within column is different statistically at $p<0.05$

THE CORRELATION BETWEEN TOTAL PHENOLIC CONTENT, DPPH FREE RADICAL SCAVENGING ACTIVITY AND FERRIC REDUCING ANTIOXIDANT POWER

It was observed that the total phenolic content of YCE and CS of different varieties correlates positively with both DPPH free radical scavenging activity and FRAP. At the highest crude extract concentration of 800 µg/mL, there was a very strong positive correlation between TPC with DPPH free radical scavenging activity (R^2=0.9899, R=0.9949) (Figure 1). While the correlation between TPC with FRAP was also well established (R^2=0.9952, R=0.9976) (Figure 2), confirming that phenolic compounds are likely

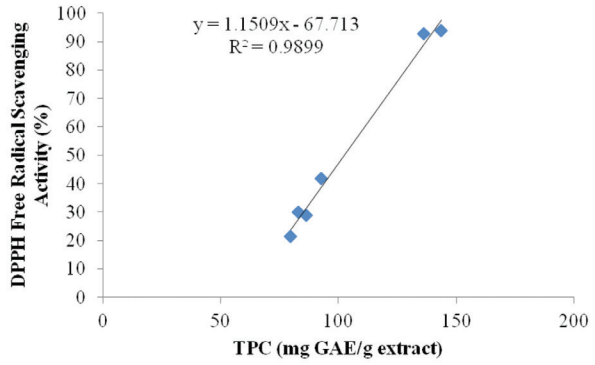

FIGURE 1. Linear correlation between the total phenolic content (mg GAE/g extract) and DPPH free radical scavenging activity (%)

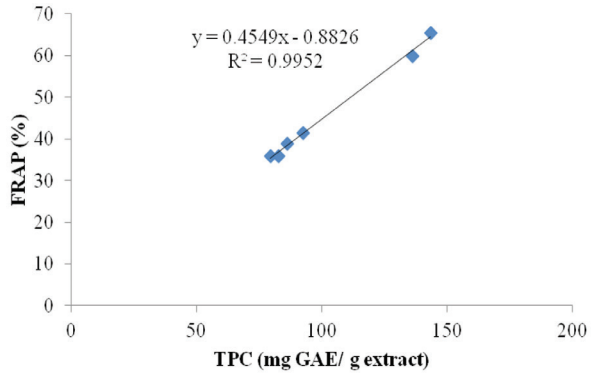

FIGURE 2. Linear correlation between the total phenolic content (mg GAE/g extract) and FRAP (%)

to contribute to antioxidant activity of YCE and CS crude extracts. Furthermore, there was also significant correlation observed between DPPH free radical scavenging activity and FRAP (R^2 =0.9735, R=0.9867) (Figure 3).

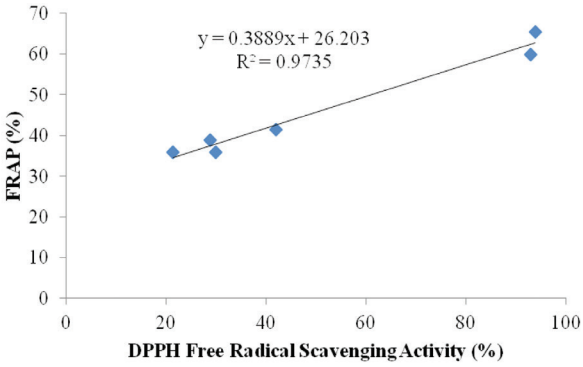

FIGURE 3. Linear correlation between DPPH free radical scavenging activity (%) and FRAP (%)

It was consistent to the result of study on fruits from Ecuador where positive correlations were obtained between phenolic content and antioxidant capacity which was measured as DPPH free radical scavenging activity, FRAP and TEAC (Vasco et al. 2008). This indicated that total phenolic content played a major role in the antioxidant activity of plant materials specifically YCE and CS.

CONCLUSION

Antioxidant capacity, total phenolic and total flavonoid of YCE and its CS are varies in percentage among different varieties. Data from the current study support the conclusion that YCE and CS tended to associated with high antioxidant capacity, total phenolic and total flavonoid content. CS was found to be more prominent antioxidant if compare to YCE. Meanwhile, difference in variety also affects the antioxidant capacity indirectly. Among different varieties of YCE and CS analysed, BiCo variety exhibited significantly higher antioxidative activities and scavenging capacities. There is significant correlation between TPC, DPPH free radical scavenging activity and FRAP of YCE and CS of different varieties.

ACKNOWLEDGEMENTS

The authors acknowledge USM's RUI fund (1001/PPSK/813057) and Fundamental Research Grant Scheme (FRGS) of Ministry of Education (203.PPSK.6171190).

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School of Health Sciences, Health Campus
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan Darul Naim
Malaysia

*Corresponding author; email: wrosli@usm.my

Received: 21 March 2015
Accepted: 6 July 2015